REMARKS

These remarks are in response to the Office Action mailed July 12, 2001 and the Advisory Action mailed February 11, 2002.

Applicants and their representatives acknowledge with gratitude, Examiner Prasad for granting the interview of March 28, 2002 and Examiner Prasad and Primary Examiner Spector for granting them the interview of April 9, 2002.

After entry of the present Amendment, claims 64-65, 70-95, 97-106, and 111-148 will be pending and under consideration. In the present communication, claims 64, 65, 70, 71, 72, 104, 105, 106, 111, 112, 120, 123, 124, 135, and 136 have been amended, and claims 137-148 have been added. Claims 66-69, 96, and 107-110 have been cancelled without prejudice. A marked up version to show the changes made is attached herewith as Exhibit A. The claims as they would stand upon entry of the amendments is attached herewith as Exhibit B.

The amendments submitted herewith are supported by the specification and original claims and do not add new matter. Amendments to claims 64 and 104 reciting that the skin nucleic acid profile after application is not affected for up to about two hours is supported by the disclosure as filed, for example at page 8, lines 7-8.

Claim Rejection Under 35 U.S.C. § 112, first paragraph

The rejection of claims 64 to 94 and 104 to 134 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement, is respectfully traversed.

Applicants invention, as defined by claims 64 and 104, and claims dependent therefrom, recites a non-invasive method for obtaining a skin sample for use in isolating or detecting a nucleic

acid in the skin sample. The method includes applying an adhesive to the skin or scraping the skin such that the adhesive or scraped skin sample contains nucleic acid from the skin, followed by isolation or detection of the nucleic acid from the skin sample. Applicants respectfully submit that the disclosure provides adequate support to enable one of skill in the art to isolate or detect any nucleic acid molecules present in a skin sample obtained using invention method.

In the Office Action of July 12, 2001, the Examiner asserts that the specification provides enablement only for detection of certain cytokines, *i.e.,* IL-4, IL-8, IL-13, iNOS and IFN-γ, in a skin sample, and states that the detection and quantitation of other nucleic acids is not supported or necessary. The Examiner also asserts that cytokines other than IL-4, IL-8, IL-13, iNOS and IFN-γ are not known to distinguish irritant contact dermatitis (ICD) from allergic contact dermatitis (ACD). Applicant notes that, the claims do not specifically recite a method of distinguishing ICD from ACD. Rather, the claims recite a method of obtaining a skin sample to use for isolating and detecting nucleic acids in the skin sample. Invention methods can be used for obtaining a skin sample by a non-invasive method for isolating or detecting nucleic acids to achieve a variety of medical and scientific goals. For example, the disclosure demonstrates that invention methods can be used to determine the presence of one or more cytokine-encoding nucleic acids, *i.e.,* a specific nucleic acid profile, thereby distinguishing ICD from ACD, for example.

Methods of the invention can also be used to obtain a nucleic acid sample from skin to determine a nucleic acid profile wherein the nucleic acids encode polypeptides that are not all cytokines. All embodiments of methods of the invention need not be provided. Indeed, the U.S. Court of Customs and Patent Appeals stated that, "there is no magical relation between the number of representative examples and the breadth of the claims; the number and variety of examples are irrelevant if the disclosure is 'enabling' and sets forth the 'best mode contemplated'." (*In re Borkowski and Van Venrooy*, 164 USPQ 642, 646 (C.C.P.A., 1970)).

Although the disclosure need not present examples wherein further nucleic acids are obtained and detected, Applicant provides factual evidence demonstrating that the disclosure enables the claimed invention in Declaration by Inventors under 37 C.F.R. § 1.132, submitted herewith. The Declaration demonstrates that a gene for glyceraldehyde-3- phosphate dehydrogenase (GAPDH) can be detected in skin samples obtained using invention methods. The GAPDH gene, is a "house-keeping gene" encoding a protein that is entirely unrelated to genes encoding cytokines or to genes that indicate an inflammatory profile, or to genes that distinguish between ICD and ACD. Thus, invention methods clearly are enabling for both cytokine and non-cytokine genes.

The Advisory Action mailed February 11, 2002, alleges that the attached declaration is insufficient to overcome the rejection of claims 64-136 under 35 U.S.C. 112, first paragraph. The Advisory Action indicates that the data presented in Table 1 of the attached declaration is not commensurate with the scope of the claims with respect to detection of IL-4 because the mRNA for IL-4 could not be detected in any samples including the control sample. As discussed in the interviews of March 28, 2002 and April 9, 2002, IL-4 was not detected in the samples of the attached Declaration because it was not present in the samples of this experiment. However, the housekeeping gene glyceraldehydes-3-phosphate dehydrogenase (GADPH) was detected in this experiment, providing support for the Applicants assertion that the methods of the present invention are generally applicable and can be used to detect any polynucleotide that is present in the sample.

The results of the attached Declaration with respect to the lack of IL-4 expression in those samples are consistent with the results reported in the specification. The samples analyzed in the attached study include control samples without irritation and samples with irritation. These results are consistent with the specification, where it is reported that IL-4 was not detected during *irritant* induced dermatosis (Specification as filed page 19, lines 18-19). In the study reported in the specification, IL-4 was only induced during allergic contact dermatosis.

In fact, this induction of expression of IL-4 during allergic contact dermatitis but not irritant-induced dermatitis underscores the enablement of the present invention not only for the general method of detecting nucleic acids in skin samples, but also in detecting specific gene products, such as IL-4, in skin samples. Levels of IL-4 in dermatitis-afflicted skin can be measured using methods of the present invention to assist in the differentiation of allergic contact dermatitis (ACD) from irritant contact dermatitis (ICD) (See specification page 19, lines 10-19).

Likewise, the detection of other specific cytokines using methods of the present invention, provide further support that the present invention enables not only the general method of detecting any nucleic acid, but also methods for detecting expression of specific genes. For example, as illustrated in Table 3 (Page 22) and discussed on page 13, lines 24-25, IL-13 is specifically increased in ACD and not ICD, indicating that like IL-4, detection of IL-13 assists in the differentiation of the type of dermatitis of the skin. Furthermore, as illustrated in the specification at page 19, lines 20-22 and discussed in the specification at page 13, lines 25-28, analysis of IL-8 levels can be used to diagnose general contact dermatitis since it is induced by an allergic or an irritant reaction.

Further support that the methods of the present invention are enabled for the specifically recited genes is provided in the art. For example, upon injury, activated keratinocytes express signaling growth factors and cytokines including IL-3, IL-6, IL-8, and GM-CSF (see Exhibit C, Freedberg et al., "Keratins and Keratinocyte Activation Cycle," *J. Invest. Dermatology*, 116, 633-40, (2001) at page 636, citing studies from 1987 and 1990). Therefore, methods which detect these cytokines provide information regarding whether skin tissue has been injured. This is further supported by Corsini et al., which indicates that damage to the skin causes production of IL-6, IL-8, and GM-CSF (Corsini et al. Toxicology Letters 102-103, 277-282 (1998)) (see Exhibit D).

Hamid et al. report that expression of IL-12 and IL-13 is increased in skin biopsies of patients with atopic dermatitis (AD) (Hamid et al., *J. Allergy Clin. Immunol.* 98, 225 (1996) (Abstract))

(Exhibit E). This provides evidence that methods of the present invention for detecting IL-12 or IL-13 assist in determining whether a patient is afflicted with AD. Junghans et al. reported that expression of IL-1 and IL-12 is upregulated in normal and affected skin of patients suffering from atopic dermatitis, even normal skin of atopic dermatitis patients (Junghans et al., *J. Invest. Dermatol.* 111, 1184-88 (Abstract) (1998)) (Exhibit G). Therefore, the methods of the present invention for detecting these cytokines provide useful information in diagnosing an individual suffering from atopic dermatitis. Yawalkar et al. reported that skin biopsy specimens from drug-induced maculopapular exanthema revealed a higher level of IL-5 than normal skin (Yawalkar et al., *Int. Arch. Allergy Immunol.* 124, 336 (2001)) (Exhibit I), thereby providing further evidence of a utility for claims of the present invention that utilize IL-5.

Ohmen et al. report that distinct cytokine profiles distinguish atopic dermatitis lesions, allergic contact dermatitis lesions, and tuberculin reactions (Ohman et al. *J. Immunol.*, 154, 1956 (Abstract) (1995)) (Exhibit F). These studies revealed over-expression of IL-10 mRNA in atopic dermatitis. Furthermore, they showed that IL-4 was most strongly expressed in allergic contact dermatitis lesions, and IFN-gamma mRNA was the predominant cytokine in tuberculin reactions. Therefore, methods of the present invention for detecting IL-10, IL-4, and IFN-gamma provide valuable information regarding the diagnosis of these pathologies. Furthermore, Ryan et al. report that steady state levels of IL-2, IL-4, and IL-10 were increased in allergen-treated sites of the epidermis versus irritant-treated or control vehicle-treated sites (*Am. J. Contact Dermatol.*, 10 (1999)) (Abstract in Exhibit H; Full article to be provided in an Information Disclosure Statement). Therefore, methods of the present invention for detecting these cytokines provide valuable information regarding the nature of a dermatitis lesion.

The Advisory Action alleged that the attached declaration was insufficient because the details of how data was pooled to obtain the reported Ct values was allegedly unclear. Regarding the concerns expressed in the Advisory Action and discussed during the interviews, each value reported

in Table 1 of the Declaration is an average of the values obtained for the three sites having the same treatment. (Declaration page 2, last paragraph). Furthermore, the data is obtained from three different sites of the same individual (See First paragraph of Materials and Methods section of page 1 "three sites were tested"; and Table 1 headings "Subject 1," "Subject 2") to determine Ct values.

The Advisory Action alleged that the attached declaration was insufficient because the reporting of Ct values out to 1/100th of a cycle is allegedly unclear. The Ct value was calculated using a real-time PCR assay, the Taqman assay (Declaration page 2, second full paragraph). The Ct value was calculated to be the time (expressed in cycles) at which a PCR reaction achieves a standard fluorescence value (Declaration Page 2, last paragraph). Therefore, Ct values are not restricted to whole number cycle counts, as would be the case if fluorescence was only measured after completion of a cycle. The applicants understand based on the interviews of March 28 and April 9, 2002 that their explanations, as outlined above, of the issues regarding the attached Declaration were accepted by the Examiners as overcoming the issues regarding the declaration raised in the Advisory Action.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 65 to 94 and 104 to 136 under 35 U.S.C. § 112, first paragraph.

The rejection of claims 66 to 69 and 104 to 115 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement is respectfully traversed.

Applicants respectfully disagree with the Examiner's assertion that there is no guidance as to the number of times tape stripping can be performed while maintaining the non-invasive character of invention method. Those of skill in the art will readily know how many times tape stripping can be performed without causing an inflammation reaction. However, in order to expedite prosecution and reduce the issues on appeal, claims 64 and 104, and claims dependent therefrom have been amended

to recite a non-invasive method for obtaining a skin sample for use in isolating or detecting a nucleic acid in a skin sample wherein the method includes applying at least one application of an adhesive to the skin in a manner such that the application does not affect the skin nucleic acid profile. Support for the amendment is found in Example 1 (at page 18, lines 8 to 10) where it is stated that the "process of tape stripping itself does not affect the skin cytokine profile during the first few hours after the procedure is done".

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 66 to 69 and 104 to 115 under 35 U.S.C. § 112, first paragraph.

Claim Rejections Under 35 U.S.C. § 102

The rejection of claims 64, 65, 70 to 82, 85 to 91 and 95 to 101 under 35 U.S.C. § 102 as allegedly being anticipated by Nickoloff and Naidu ((1994) *J. Am. Acad. Dermatol.*, 30:535-546) is respectfully traversed.

Applicants' invention methods are directed to a non-invasive method of obtaining a skin sample containing nucleic acid. The method, as described by amended claim 64 and claims dependent therefrom, includes applying and removing an adhesive surface to the skin such that nucleic acid adheres to the adhesive surface after its removal from the skin and in a manner such that the adhesive application does not affect the skin nucleic acid profile. Invention methods are designed to obtain a skin sample without causing an inflammatory response in the skin cells. In fact, tape stripping, as performed by the method of the invention does not affect the skin cytokine profile and no inflammatory cells migrate from the circulation into the dermis or epidermis during the first few hours after the procedure is performed (Specification, page 18, lines 8 to 11).

In contrast, Nickoloff and Naidu disclose using "tape-stripping" to cause epidermal hyperplasia, *i.e.*, irritation to the skin, following which skin specimens are collected by punch biopsy.

An invasive procedure such as a punch biopsy typically requires the removal of a small cylinder-shaped piece of tissue and when a large skin sample is obtained by punch biopsy, the area may need to be closed with stitches. Thus, the tape-stripping procedure used in Nickoloff and Naidu is specifically designed to cause an inflammatory reaction that is exacerbated by the performance of a punch biopsy and possibly stitches. Once the tape strips are used to cause irritation to the skin, the tape strips are discarded and have no analytical role in the study. Nickoloff and Naidu do not disclose or suggest using tape-stripping to obtain skin samples. Indeed, by following their tape-stripping procedure with a punch biopsy procedure to obtain skin specimens, Nickoloff and Naidu teach away from obtaining skin samples using tape or any other adhesive surface. Therefore, Nickoloff and Naidu cannot anticipate Applicants' invention. Accordingly, reconsideration and withdrawal of the rejection of claims 64, 65, 70 to 82, 85 to 91 and 95 to 101 under 35 U.S.C. § 102 is respectfully requested.

Claim Rejections Under 35 U.S.C. § 103

The rejection of claims 64 to 65, 70 to 74, 76 to 82, 85 to 91, 93, 96 to 101 and 103 under 35 U.S.C. § 103(a) as being allegedly obvious over Molen *et al.* (van der Molen *et al.* (1997) *Arch. Dermatol. Res.* 289:514-518, (hereinafter "van der Molen") in view of Kondo *et al.*(1994) L*ymphokine Cytokine Res*, 13:367-375, hereinafter "Kondo") is respectfully traversed.

Applicants' invention is directed to a non-invasive method for obtaining a skin sample from which nucleic acid can be isolated or detected. The method includes applying and removing at least one application of an adhesive to the skin such that a skin sample adheres to the adhesive after its removal from the skin.

In contrast, van der Molen discloses a study related to the kinetics and penetration depth of drugs wherein tape stripping is used to remove skin cells so that the skin remaining subsequent to tape stripping can be examined using morphological and histological methods. As many as 40

rounds of tape stripping are performed (Abstract) which is likely to result in an invasive, inflammatory response. The tape strips obtained in van der Molen were examined using X-ray microanalysis for the sole purpose of determining the distribution of skin over the tape surface in order to assess the efficacy of tape-stripping in removing skin from skin furrows. Van der Molen does not disclose or suggest using tape-stripping to obtain skin samples that can be used for isolation or detection of nucleic acids. Moreover, van der Molen does not disclose or suggest using the skin samples obtained by tape-stripping for any purpose other than evaluating the distribution of a marker compound in the skin. Accordingly, van der Molen does not disclose or suggest Applicants' invention.

The deficiencies of van der Molen can not be remedied by further reliance on Kondo. Kondo discloses a cytokine profile in mouse ear epidermis following allergic and irritating stimuli. In Kondo, epidermis samples are obtained following animal sacrifice by dissecting the animal ears away from the animal and incubating the ears with enzyme solution for 24 hours at 4°C, after which the epidermal sheet can be peeled from the ears (page 368, column 2). Thus, Kondo discloses a tedious and invasive procedure requiring the death of the subject to obtain skin samples. Kondo does not disclose or suggest using a non-invasive method to obtain skin specimens. Accordingly, Kondo does not disclose or suggest Applicants' invention.

Kondo does not remedy the failures of van der Molen and van der Molen does not remedy the failures of Kondo to disclose or suggest Applicants' claimed non-invasive methods. Indeed, the references may not be combined because the proposed modification to the prior art renders the prior art unsatisfactory for its intended purpose. (*In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984; quoted in MPEP § 214301: "If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification."). The tape strips obtained by van der Molen were examined by X-ray microanalysis in combination with scanning electron microscopy (SEM) (van der Molen, page

515, column b, third paragraph). These histological techniques require that the tape strips with adhering cells be cut to size, and coated with carbon for x-ray analysis, followed by sputter-coating in gold for analysis by SEM. Such treatment of the tape strips, designed to preserve the morphological features of the cells, is incompatible with molecular methods for isolating or detecting nucleic acids in the cells. Thus, the combination of the prior art references renders the prior art method unsatisfactory for its intended purpose and therefore, the references may not be combined.

Moreover, neither van der Molen nor Kondo provides any suggestion or motivation to combine the respective references. van der Molen discloses tape stripping as a means of obtaining cells only for the purpose of histological analysis. No molecular analysis, *e.g.,* detection or quantitation of nucleic acids in skin cells, is disclosed or suggested. Kondo discloses analysis of nucleic acids in skin samples that are collected by an invasive procedure requiring the sacrifice of the subject animal. Kondo does not disclose or suggest any other means of collecting skin samples.

Accordingly, Applicants respectfully submit that neither van der Molen, nor Kondo, either separately or taken together, renders obvious the present invention. Therefore, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

In the event any matters remain to be resolved in view of this communication, Examiner is requested to telephone Lisa A. Haile, J.D., Ph.D. at (858) 677-1456 so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: May 13, 2002

Lisa A. Haile, J.D., Ph.D.
Reg. No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

CUSTOMER NUMBER 28213
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

Enclosures: Exhibits A through I
Declaration Under 37 C.F.R. § 1.132

EXHIBIT A: CLAIMS WITH MARKINGS TO SHOW CHANGES MADE

In the Claims

Please cancel claims 66-69, 96, and 107-110 without prejudice.

Please amend the claims as follows:

64. (Amended) A non-invasive method for obtaining a skin sample for use in isolating or detecting a nucleic acid in [a] the skin sample, the method comprising:

(a) applying at least one application of an adhesive to the skin and removing the adhesive from the skin in a manner such that the skin nucleic acid profile after application is not affected for up to about two hours and such that a sample comprising a nucleic acid adheres to the adhesive after its removal, or, scraping the skin with an instrument to remove a sample comprising a nucleic acid from the skin, thereby obtaining a skin sample comprising a nucleic acid; and

(b) isolating or detecting the nucleic acid from the skin sample of step (a).

65. (Amended) The method of claim 64, wherein the skin sample [consists essentially of] comprises at least one of stratum corneum cells, stratum lucidum cells, stratum granulosum cells, stratum spinosum cells, and stratum basilis cells, or any combination thereof.

70. (Amended) The method of claim 64, wherein [an adhesive surface is applied one time to the skin] the skin sample is isolated by applying the adhesive surface to the skin between one and twenty five times to obtain the skin sample.

71. (Amended) The method of claim [70] 64, wherein [an adhesive surface is applied two or more times to the skin] the skin sample is isolated by applying the adhesive surface to the skin between one and two times to obtain the skin sample.

72. (Amended) The method of claim [65] 64, wherein the [stratum corneum skin] sample is isolated by one application of an adhesive surface to an outer layer of the skin.

82. (Twice amended) The method of claim [79] 80, wherein the cytokine comprises interleukin-1 (IL-l), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), [interleukin-7 (IL-7),] interleukin-8 (IL-8), [interleukin-9 (IL-9),] interleukin-10 (IL-I0), interleukin-12 (IL-12), interleukin-13 (IL-13), [interleukin-14 (IL-14),] granulocyte macrophage colony stimulating factor (GM-CSF), or an interferon, or any combination thereof.

104. (Amended) A non-invasive method for obtaining a skin sample for use in isolating or detecting nucleic acid encoding a cytokine in the skin sample, the method comprising:

applying [at least one application and of] an adhesive surface to the skin and removing the adhesive surface from the skin such that a skin sample comprising nucleic acid in an amount sufficient for subsequent isolation or detection adheres to the adhesive surface after its removal and in a manner such that the skin nucleic acid profile after application is not affected for up to about two hours, thereby obtaining a skin sample for use in isolating or detecting a nucleic acid in a skin sample.

105. (Amended) The method of claim 104, wherein the skin sample [consists essentially of] comprises at least one of stratum corneum cells, stratum lucidum cells, stratum granulosum cells, stratum spinosum cells, and stratum basilis cells, or any combination thereof.

106. (Amended) The method of claim 105, wherein the [stratum corneum] sample is isolated by one application of an adhesive surface to an outer layer of the skin.

111. (Amended) The method of claim 104, wherein [the at least one application is one application] the skin sample is isolated by applying an adhesive surface to the skin between one and twenty five times.

112. (Amended) The method of claim 104, wherein the [at least one application is two or more applications] the skin sample is isolated by applying an adhesive surface to the skin between one and two times.

123. (Amended) The method of claim 121, wherein the cytokine is interleukin-1 (IL-l), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), [interleukin-7 (IL-7),] interleukin-8 (IL-8), [interleukin-9 (IL-9),] interleukin-10 (IL-I0), interleukin-12 (IL-12), interleukin-13 (IL-13), [interleukin-14 (IL-14),] granulocyte macrophage colony stimulating factor (GM-CSF), or an interferon or any combination thereof.

124. (Amended) The method of claim [120] 121, wherein the cytokine is an inflammatory mediator.

135. (Amended) A non-invasive method for obtaining a skin sample for use in isolating or detecting nucleic acid in the skin sample, the method comprising:

scraping the skin with an instrument to remove a skin sample comprising nucleic acid in an amount sufficient for subsequent isolation or detection, and in a manner such that the skin nucleic acid profile after application is not affected for up to about two hours, thereby obtaining a skin sample for use in isolating or detecting a nucleic acid in a skin sample.

136. (Amended) A non-invasive method for obtaining a skin sample for use in isolating or detecting a nucleic acid in a skin sample, the method comprising:

(a) scraping the skin with an instrument to remove a sample comprising a nucleic acid from the skin, and in a manner such that the skin nucleic acid profile after application is not affected for up to about two hours, thereby obtaining a skin sample comprising a nucleic acid;

(b) isolating or detecting the nucleic acid from the skin sample of step (a).

Please add the following claims:

--137. The method of claim 80, wherein the cytokine is IL-1.

138. The method of claim 80, wherein the cytokine is IL-2.

139. The method of claim 80, wherein the cytokine is IL-3.

140. The method of claim 80, wherein the cytokine is IL-4.

141. The method of claim 80, wherein the cytokine is IL-5.

142. The method of claim 80, wherein the cytokine is IL-6.

143. The method of claim 80, wherein the cytokine is IL-8.

144. The method of claim 80, wherein the cytokine is IL-I0.

145. The method of claim 80, wherein the cytokine is IL-12.

146. The method of claim 80, wherein the cytokine is IL-13.

147. The method of claim 80, wherein the cytokine is GM-CSF.

148. The method of claim 80, wherein the cytokine is an interferon.--